IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE:	U.S.S.N. 09/518,165	)
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FILED:	March 1, 2000	)
		) GROUP ART UNIT: 1641
		)
INVENTORS:	V.A. Koulchin , N.J. Moore, E.V. Molokova and M.K. Fent	)
		)
		) EXAMINER: Ja-Na Hines
		)
TITLE:	METHOD FOR DETECTING THE PRESENCE OF TARGET BACTERIA OR A TARGET COMPONENT CARBOHYDRATE ANTIGEN THEREOF	)

DECLARATION

I, William J. Palin, being duly advised that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements and the like may jeopardize the validity of the application or of any patent resulting therefrom, solemnly declare that all of the following statements made herein of my own knowledge are true and all of the following statements made on information and belief are believed to be true:

On that basis, I declare as follows:

1. I am presently Vice President of Research at Binax, Inc., assignee of the captioned patent application. I joined Binax, Inc. in 2001, having already spent some 35 years actively engaged in immunology research and development.

2. More specifically, after receipt in 1966 of my B.A. degree from Providence College, Providence, R.I. where I majored in Biology, I joined the 6th U.S. Army Medical Laboratory at Fort Baker, CA as a Biological Sciences Assistant and was immediately assigned to the Adenovirus Surveillance Project. During my three years there, I primarily worked on that project, but I also developed a new indirect fluorescent antibody assay for detecting Rubella antibody in patient samples.

3. In 1969, I entered the University of Minnesota at Minneapolis in the Department of Microbiology as a Research Assistant and graduate student. My research, on which I wrote a thesis entitled "Endotoxin Immunity" involved defining the structure and function correlates and the immunological specificity in the Lipid A portion of this lipopolysaccharide, and I also developed a radioimmunoassay to study antibody reactivity to Lipid A. In 1975 I received the Ph.D. degree and during 1976-1977 I did postdoctoral work, as an NIH Fellow, in the Department of Medicine at UCLA. During this period, I developed a method for monitoring endotoxemia in mammals using a sensitive gas chromatographic-mass spectrometry assay for β-hydroxymyristate.

4. Following my postdoctoral work, I have held several positions with major companies, as Senior Scientist initially, and later, as Assistant Director and as Director of Research and Development, in which I have worked on and/ or supervised the development of a variety of immunoassays for various purposes. I commenced work on assays for bacterial carbohydrate antigens while employed at Abbott Laboratories in 1977 and have continued work involving various assays of that type throughout my career. I have also worked extensively on assays for antibodies, enzymes, viruses and other substances.

5. I have read U.S. Patent Application Ser No. 09/518,165 and each of the applications Ser No. 09/139,720, 09/397,110, and 09/485,998 referred to therein. I am familiar with the Binax immunochromatographic assays for *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* which are sold under the trademark NOW and are respectively described in U.S. Patent Applications Serial Nos. 09/139,720 and 09/397,110. I am also familiar with the records of work done on these assays, each of which depends on detecting a characteristic carbohydrate antigen of the bacterium in a fluid from a human patient.

6. I have also carefully read the Office Action dated March 20, 2003 and I have been asked to comment upon the validity and practicality of various evidentiarily unsubstantiated assumptions appearing therein. I have also been supplied with and carefully read the seven-page document "Critical Synergy: The Biotechnology Industry and Intellectual Property Protection, Presentation at the

October 17, 1994 Hearing of the U.S. Patent and Trademark Office, San Diego, CA" allegedly published by the Biotechnology Industry Organization, Washington, D.C., pages 100-107 cited at page 6 of the Action. I could find nothing in this document about immunoassays, antigens, antibodies, bacteria or immunology and see nothing in the Office Action that justifies any attempted analogy between this application Serial No. 09/518,165 and the subject matter that the document *does* discuss.

7. The Office Action ignores that there was a vast body of literature available as of March 1, 2000 when Application Ser. No. 09/518,165 was filed that pertains to the various *facts* concerning immunology that underlie this application. The existence of this body of literature, which comprises published articles, dissertations, lectures and other writings from persons located worldwide that are easily locatable through searches of widely available data bases (such as the National Library of Medicine's Pub Med and Biosis ~~515~~™, but by no means limited thereto), patents from various countries, literature from suppliers of equipment and reagents which is frequently highly informative about the capabilities and capacities of equipment, the compatibility and conditions of use of reagents, etc. For example, Polysciences, Inc. puts out a catalog that devotes some eight pages to the variations in linkage technology that may be availed of to link a ligand to an affinity chromatography column and another eight pages to the various affinity matrices it has available. For another example, Sigma Chemical Co. lists 30 different affinity matrices in its catalog and makes

available to customers, including their researchers, technical services employees highly knowledgeable about these matrices who will consult with customers by telephone, e-mail or fax about the properties of the matrices, and if needed, will recommend the one best adapted to a specific task, as well as provide assistance in solving or avoiding various problems associated with particular matrices.

8. In particular, this vast body of literature includes writings describing previous work in which carbohydrate antigens indigenous to the bacteria that are known to cause infectious diseases were identified and methods for separating them from bacterial cultures were described. The literature also includes many articles relating to methodology for minimizing the protein content of carbohydrate antigens. The literature likewise describes many uses of affinity chromatography for antibody purification.

9. At Binax, bacteria for culturing and for antibody induction in host animals is almost always obtained from Centers for Disease Control or from one of the established depositories of biological materials, such as American Type Culture Collection or Pasteur Institute. These bacteria are provided to Binax with information specifying their species and serotype and giving recommendations for their culture which include recommendations as to culture medium and other growing conditions. Laboratories such as that at Binax and the laboratories of other companies where I have previously been employed (which include Abbott, Becton Dickinson and Idexx Corporation) conduct research that is targeted toward developing useful and operable products for sale. Such laboratories are

geared toward accepting and following the well-documented observations of product and equipment suppliers about details of how to use and otherwise treat the supplied materials, while focusing upon the aspects of their research and development that are important to developing a useful diagnostic test.

10. The invention described in Application Ser. No. 09/518,165 involves the discovery that crude antibodies to bacterial carbohydrate antigens acquire greatly enhanced specificity and sensitivity toward the corresponding antigen, whether that antigen is present as a part of a whole or partial bacterium or is in free form in a human bodily fluid, if the crude antibodies have been affinity purified by passing them over a chromatographic affinity column to which has been coupled an embodiment of the target antigen that is essentially protein-free (i.e contains not more than 10% protein and preferably less).

11. In my opinion, this invention can readily be practiced by a person of ordinary skill in the art by simply following the teachings of the application and combining those teachings with prior art teachings, especially those available as of the application filing date, March 1, 2000, which relate to the particular bacterium and its characteristic carbohydrate antigens. In other words, application 09/518,165 in its examples describes exactly how the invention is applied to (1) obtain an essentially protein-free carbohydrate antigen of *Haemophilus influenzae* type b, (2) couple that essentially protein-free carbohydrate antigen to a chromatographic affinity column, (3) pass crude antibodies to *H. influenzae* type b over the column and (4) use the thus treated

antibodies in an ICT assay for the crude carbohydrate antigen of which the essentially protein-free carbohydrate antigen employed in step (2)is an embodiment. The incorporated by reference examples of Serial No. 09/139,720 describe how an essentially protein-free carbohydrate antigen of *Legionella pneumophila* serogroup 1 was obtained from a culture of those bacteria, how the essentially protein free carbohydrate antigen was coupled to a chromatographic affinity column, how crude antibodies to the bacteria were passed over the column and rendered antigen-specific and how the treated antibodies were then utilized in an ICT assay which is currently and has since September 1998 been sold for use as a diagnostic tool by Binax, Inc., under the trademark NOW®, to detect the crude carbohydrate antigen in human bodily fluid samples. The incorporated by reference examples of Serial Nos. 09/156,486 and 09/397,110 describe how an essentially protein-free embodiment of the cell wall carbohydrate antigen indigenous to all serotypes of *S. pneumoniae* was obtained from a bacterial culture and coupled to a chromatographic affinity column, how crude antibodies to the bacteria were passed over the column and rendered antigen-specific and how the treated antibodies were then employed in an ICT assay which is currently and has since August 1999 been sold for use as a diagnostic tool by Binax, Inc., also under the NOW ® trademark.

12. Each of the three assays referred to in ¶11 hereof commenced with the recognition of the need for a highly sensitive, fast and easily usable assay that would permit rapid diagnosis of a specific disease state and with an examination of what the prior literature showed with respect to efforts to produce these tests and the target antigens employed in them. In each case tentative selection of a target carbohydrate antigen was made based on known characteristics of the disease and/or the antigen. This is described on page 11, middle paragraph, of application Serial No. 09/518,165. A similar practical selection, using assistance from the literature, was followed at Binax in selecting target carbohydrate antigens for diagnostic assays relating to other bacteria-caused diseases now being developed there. The application at page 11, middle paragraph, and page 12, first full paragraph, would lead any person of ordinary skill in immunology who wished to devise an assay enabling diagnosis of a particular bacteria-caused disease state to consult the existing literature as an aid to selecting a bacterial carbohydrate target antigen for the assay.

13. Application 09/518,165 also clearly teaches that, for separating the selected target carbohydrate antigen from a bacterial culture, one should consult the literature. See p.14, lines 3-5 of the specification and also p.25, last paragraph. These teachings about referring to the literature show that what is already known clearly also applies to rendering the carbohydrate antigen "essentially protein-free" as that term is defined in the application. It is noted that it is also well within the ordinary skill of the art in immunology to adapt product

separation and protein minimization techniques that have been published for a related carbohydrate antigen to a selected target antigen having similar functionality or composition.

14. The specification of Serial No. 09/518,165, including the information incorporated by reference, contains specific teachings about the coupling of the "essentially protein-free" carbohydrate antigen to a suitable affinity matrix, and the passage of raw antibodies thereover. A person of ordinary skill in immunology with such additional help from the literature and/or a supplier of affinity matrices as might be desired, would have no difficulty in performing this step efficaciously.

15. The specification of Serial No. 09/518,165 including the information incorporated by reference, describes in detail the preparation of an immunochromatographic strip with a "capture line" of immovable affinity purified antibodies striped across the strip near the end most remote from sample introduction and a movable deposit of gold-labelled affinity-purified antibodies placed near the point of sample introduction. The specification teaches that other labels may be used in lieu of gold. It also cites references that teach modes of conjugating gold to antibodies and other ligands. A great deal of technical literature about labels and methods for conjugating them to ligands exists. The teachings in the application, including those incorporated by reference about the preparation of the ICT strips for detecting target carbohydrate antigens of *Legionella pneumophila* serogroup 1 and *Streptococcus*

pneumoniae, are informative and, in my opinion, could readily be followed by a person of ordinary skill in immunology, working with antibodies raised to either the host bacteria of the antigen or an Ig G cut of the selected carbohydrate antigen thereof and then affinity-purified as described in application 09/518,165, to arrive at a useful and potentially highly successful diagnostic test.

16. With specific reference to the office action:

(a) Page 6, 2nd paragraph, states. "For example, the specification at page 14 teaches different purification of carbohydrate antigen steps, including an incubation step, sonication steps, repeated precipitation and centrifugation steps, lyophilization, subjected to Lowry assay and for proteins and tested for carbohydrates by phenol-sulfuric acid method."

First of all, this listing is taken from the specific exemplification relating to *Haemophilus influenzae* type b. Secondly the only "step" listed that pertains to purification of antigen *per se* is the "repeated precipitation and centrifugation" step. Thirdly, there is no "sonication" step referred to on page 14 of the specification of Application Serial No. 09/518,165. Perhaps most importantly, I understand the disclosure of this application to teach that any known method of obtaining the desired antigen from a culture of the bacteria may be used, so long as care is taken to ensure that the antigen is recovered in essentially protein-free form as defined therein--i.e., containing not more, and preferably less, than 10% of protein by weight.

(b) The action then states:

> "It is well known in the art that specific bacterial species require specific extraction methods, yet the claims do not take this into consideration and generically claim a method of detection".

I understand that the Examiner is here speaking of "extraction" of the antigen from bacteria *contained in a sample* that is to be assayed according to the general ICT method recited in certain of the claims. The premise that antigen must be extracted from bacteria contained in a sample of human bodily fluid, however, is inconsistent with the experiences of Binax in that Binax has found it to be unnecessary to take steps to "extract" the carbohydrate antigen from bacteria in such samples in order to run and complete a satisfactory assay.

Further, the many entities that have purchased and satisfactorily used the Binax NOW® tests for *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* have successfully performed well over a million assays on human bodily fluids without manifesting any need to "extract" antigen from bacteria contained in human fluids.

(c) I have been advised by counsel that the Examiner's position relating to "extraction" probably relies upon the teaching of Imrich *et al* U.S. patent 5,415,994 wherein "an extraction chamber" is provided and the sample on a swab is delivered to this zone and treated with an "extraction solution" prior to the assay of the sample. See col.2, lines 26-40; see also col.1, l.67 to col.2, l.9 wherein prior art is identified that apparently alludes to the alleged need for "extraction" in order for optimal detection of the bacterium by monoclonal antibodies. It is not clear whether the Imrich *et al* group actually ever worked with liquid samples, even though these are mentioned at col.3, lines 11-13 as being operable in the method disclosed. "Pharyngeal exudates", to which reference is made at col.1, l.55; col.3, lines 12-

13 and col.4, line 16 appear to be of special concern in this patent. A "pharyngeal exudate" is often a mass of highly viscous semi-solid material that has lodged in the nose or throat and has not passed through human organs that are now known to destroy the cell walls of bacteria, such as the kidney and /or the liver, or been subjected to the action of stomach acids or digestive enzymes, both of which also "open up" bacteria. Pharyngeal exudates are also often characterized by substantial presence of live bacteria which are growing and reproducing and are sometimes harder to "open" than the partial fragments of bacteria prevalent in blood, urine, spinal fluid and other bodily liquids. It is further noteworthy that Example 2, the only specific example of an actual assay, was conducted on killed *whole* Group A *Streptococcus* bacteria, which at least impliedly had intact cell walls. In short, the Imrich *et al* patent, as I understand it, says nothing inconsistent with the Binax experience that *no* "extraction" of bacteria contained in human or other mammalian *liquid* samples, (which are the samples of choice to be used in the assays covered by the present application) is necessary.

I also note that *Streptococcus pyogenes* (commonly called Strep A) is well known to immunologists as requiring a more rigorous and harsh "extraction" treatment than most bacteria. This treatment is normally made with ~~mono~~micro nitrous acid at pH2, in order to expose its target carbohydrate antigen to recognition by antibodies. Accordingly, this bacterium cannot be relied on for purposes of generalization in respect of need for "extraction" of samples suspected of containing target antigens. By contrast, such bacteria as, e.g. *Streptococcus pneumoniae* and various *Haemophilus* species are well-known to undergo spontaneous lysis or splitting of cell walls. This spontaneous lysis is also known as autolysis.

Still further, I note that in instances where extraction may be needed because of the occurrence in samples of bacteria having intact cell walls, the science of immunology, as applied to disease causing bacteria, is so well-developed that the literature contains a substantial body of information about how particular bacteria species should be "extracted" when this is found to be needed. This literature is well known and readily available to immunologists generally. An immunologist wishing to follow the process of Application Serial No. 518,165 and develop an ICT test for detecting a carbohydrate antigen of a bacterium likely to possess intact cell walls when present in a bodily fluid would normally be aware of this problem at the commencement of the development work and would have determined from the literature the nature of the cell wall splitting reagents likely to be needed in an actual test protocol.

(d) I state unequivocally that the disclosure at page 20 in Section C "Immunoassay Procedures" of Application Serial No.09/518,165 of the addition of three drops of "Reagent A" consisting of Tween 20, sodium azide and sodium dodecyl sulfate in sodium citrate phosphate buffer to the sample does not have the purpose to "produce the crude carbohydrate antigen" as alleged in at page 8 of the Office Action and does not produce that result. In the Binax NOW ® ICT tests and in Application Serial No. 09/518,165, "Reagent A" has a dual function. Typically, the volume of liquid sample is not sufficient to ensure that the sample will flow through the immunochromatographic medium. The addition of Reagent A augments the sample volume sufficiently to ensure that flow occurs with enough momentum to pick up and carry with it the labelled antibodies that have been movably deposited in the flow path. Secondly, the components of this Reagent A are a buffer, salts and surfactant. The

buffer and salts provide a milieu that facilitates the reaction of antigens in the sample with labelled antibodies that occurs during flow and the further reaction of labelled antibody-antigen reaction products with immobilized antibodies that occurs at the capture line. The surfactant present has the function of reducing the non-specific binding that often occurs during tests.

(e) The Office Action's reference to the different "Reagent A"--i.e., tris base containing the zwitterionic detergent SB3-8--is unrelated to any requirement of Application Serial No. 09/518,165. First of all, "Reagent A" is a generic term that Binax applies to *any* additive it places in a commercial assay kit. The term "Reagent A" is not directed to the composition or purpose of the additive. It is simply a convenient term. The particular purpose of the Reagent A additive selected for a particular test is always addressed in the leaflet included in the specific kit for that test. Secondly, Application Serial No. 09/458,998 involves use of the same affinity-purified antibodies described in Serial No. 09/139,720–but in an enzyme immunoassay for *Legionella* bacteria, especially *Legionella pneumophila* serogroup 1, in environmental water. Application Serial No. 09/458,998 hence is *not* directed to the same process invention and ICT test protocol that appears in each of applications Serial Nos. 09/139,720, 09/156,486, 09/397,110 and 09/518,165. Rather, Serial No. 09/458,998 involves a different use for the affinity-purified antibodies of *Legionella* bacteria from that of detecting in a fluid from an infected human patient a carbohydrate antigen that enables identification of the bacterium to which that antigen is indigenous as the agent causative of the patients disease state. In application Serial No. 09/458,998, bacteria to be assayed have been growing in water in old pipes and other structures of heating/air-conditioning systems, in pools of stagnant water, and in other still water. These bacteria have intact cell walls and in some cases are also

contained within strong husklike structures. To assay for the crude carbohydrate antigen of such bacteria, the bacteria do often need to be opened up, either mechanically or by an extraction agent or both. Such opening of cell walls is the function of the tris base/SB3-8 reagent in application 09/458,998.

(f) The function of this latter tris base/SB3-8 reagent relative to enabling an assay for the presence of *Legionella* bacteria present in environmental water is *not* related to how one may "achieve purification of an essentially protein-free carbohydrate antigen" as implied in the last sentence on page 8 of the office action. Rather, the tris base/SB3-8 reagent is used to open up or aid in opening up the cell wall and/or the husklike structure of *Legionella* bacteria present in environmental water so that the target carbohydrate antigen is available to be detected by the reagents used to perform the enzyme immunoassay described in Application Ser. No. 09/458,998. No such opening up is needed to perform the described ICT assays of Applications Ser. Nos. 09/139,720; 09/156, 486; 09/397,110 or 09/518,165, in each of which the substance to be assayed is a human bodily fluid.

(g) The Office Action at page 9 states "the instant claims fail to distinguish between detecting gram negative and gram positive bacteria by separate method steps". The suggestion, apparently, is that the methodology for detecting gram positive and gram negative bacteria in an ICT immunoassay should somehow involve different method steps. But application 07/139,720 shows how, by selecting a carbohydrate antigen known to be characteristic of *Legionella pneumophila* serogroup 1, obtaining it in essentially protein-free form, applying it to an affinity column, purifying crude antibodies raised either to the bacteria or to tan Ig G cut of the same antigen by passing the antibodies over the purified antigen on the

column and using the thus purified antibodies in the described ICT test to detect the crude antigen in a test sample of human bodily fluid, the presence of the bacterium is necessarily detected. *Legionella pneumophila* serogroup 1 is a Gram stain negative bacterium as the office action appears to concede at page 15. Application Ser. No. 09/397,110 and its parent application teach employing *the same* steps commencing by obtaining the essentially protein-free carbohydrate antigen--this one known to inhere in all serotypes of *Streptococcus pneumoniae*--followed by applying the essentially protein-free antigen to a chromatographic affinity column, passing antibodies raised to either *S. pneumoniae* or an Ig G cut of the crude carbohydrate antigen over the column to purify them and using the antibodies in the same ICT test configuration to detect the same carbohydrate antigen in crude form in a test sample of human bodily fluid. As page 15 of the office action indicates, *Streptococcus pneumoniae* is a Gram stain positive bacterium. The process covered by application Ser. No. 09/518,165 obviously applies to both gram negative and gram-positive bacteria and as such, it is not intended to and does not "distinguish between detecting gram negative and gram positive bacteria by separate method steps" as Office Action (p.9) implies it should. The process instead is one that is generic to bacteria. By selecting a target antigen specific to a particular species or serogroup of a species of bacteria, obtaining the antigen in a form containing not more and preferably less than 10% protein, using this form of antigen to affinity-purify antibodies raised either to the bacteria or an Ig G cut of the crude antigen, incorporating the affinity purified antibodies in the ICT test strip format as described in Application Ser. No. 09/518,165 and then using the ICT test strip to assay a bodily fluid sample from a person suspected of harboring a disease caused by the bacteria, one can identify the same antigen in its

crude form, if present in the sample, with very high specificity and can accordingly diagnose and prescribe for the disease speedily. On the other hand, if the assay is negative for the target antigen, disease caused by the bacteria species (or serogroup of a species) to which the target antigen is specific can speedily be ruled out.

(h) The Office Action, p.9, states

> "The broad and generic claims encompass detecting at least species or serogroup of any bacteria, thereby including detecting multiple species in the manner claimed. If applicant does not intend for the claims to encompass such the applicant should narrowly tailor the claims to only encompass what is taught and supported by the instant specification".

This office action statement is very confusing and contradictory. The claims are intended to cover detecting bacteria species where the target carbohydrate antigen selected is characteristic of an entire species, *or* a serogroup of a species, in instances where the target carbohydrate antigen selected is characteristic *only* of a serogroup of a species. What the application teaches is a generic method for detecting a carbohydrate antigen that is characteristic of either a species of bacteria, *or else* a serogroup of a species of bacteria. The method, as already stated, involves (1) obtaining from a culture of the bacteria the target antigen of the detection in a form containing no more than 10%, and preferably less than 10%, of protein, (2) conjugating that antigen containing no more than 10% protein to a chromatographic affinity column, (3) affinity purifying antibodies (raised against either the bacteria or against an Ig G cut of the crude antigen) by passing said antibodies over the affinity column to which the antigen containing no more than 10% protein is conjugated; (4) conjugating one portion of the antibodies thus purified to a label and depositing the label-antibody conjugate movably on an ICT strip near the site of sample introduction; (5) immovably striping another portion of the

antibodies thus purified across the ICT strip near the end opposite to the site of sample introduction and (6) performing an assay by introducing a sample of human bodily fluid to the strip and allowing the sample to flow laterally along the strip so that it picks up the movably deposited label- antibody conjugate and flows therewith to the immovably striped line of unlabelled antibodies and (7) observing whether a color forms along the immovable stripe, denoting the formation of a "sandwich" of labelled antibody-antigen from sample-fixed antibody and therefore the presence of target antigen in the sample.

If the quotation from the Office Action intends to suggest that the ICT strip *could* have multiple movable deposits of *different* labelled affinity purified antibodies and multiple immovable stripes of affinity-purified antibodies so selected that each movable deposit contains purified antibodies different from each other, but identical to the antibodies in one of the immovable stripes so that a single sample of human bodily fluid could be simultaneously assayed for the presence or absence of multiple carbohydrate antigens, each such antigen being characteristic of a different bacteria species or a different serogroup of another species, this is entirely possible and even desirable within the scope of this invention. Such an assay may have the attribute of permitting a physician to use one ICT test on one sample to rule out certain bacteria as causative of a patient's disease state while also locating the one species, or serogroup of a species, of bacteria that *is* causative. In such a test for target carbohydrate antigens of multiple bacteria species, target carbohydrate antigens of multiple serogroups of bacterial species, or some combination of target carbohydrate antigens of some bacteria species and target carbohydrate antigens of serogroups of some bacteria species, it is of no importance which bacteria are gram-negative and which bacteria are gram-positive. What is important is

that each target carbohydrate antigen be characteristic of only the bacteria species, or only the serogroup of a species, in which it is found, so that whether the tests be run separately or simultaneously on the same strip, a negative test will mean that the bacteria species, or serogroup of a bacteria species, of which the target antigen is characteristic is not present in the patient sample and is not responsible for the patient's disease state, while a positive test will indicate that the bacteria species, or serogroup of a bacteria species, of which the target antigen is characteristic *is* present in the sample and (provided this presence is consistent with the patient's clinical symptoms), is highly likely to be causative of the disease state. If the quoted statement is intended to suggest that some particular carbohydrate antigen exists which is characteristic of both one or more gram-positive and one or more gram-negative bacteria, however, that is contrary to present knowledge in the art and not encompassed by application Serial No. 09/518,165.

(i) The office action at p. 9 further states:

> "Applicants statements that specific sample types perform extraction on some bacteria only bolsters the examiner's position that broad generic techniques cannot apply to all types of bacteria in any type of sample."

This statement is very hard to understand. I am informed and I believe that "Applicants" have not stated that *any* "sample types" perform extraction on bacteria. Furthermore, types of samples to be assayed do *not* and cannot "perform extraction" on the bacteria or bacteria fragments they may contain. I am further informed that Applicants *have* stated that the only instance in any presently-pending or issued Binax patent application where "extraction"of bacteria is important to enable the successful and accurate performance of an assay is the one

described in pending Application Ser. No. 09/458,998 wherein the living *Legionella* bacteria growing in environmental water, which have strong intact cell walls and in some cases, husk-like covers as well, must often be mechanically macerated and/or treated with tris base containing SB3-8, a zwitterionic detergent, in order to open their carbohydrate antigens up to the action of affinity-purified antibodies. In environmental water, however, the ICT test which is the last step of the generic process of the present invention and which was designed and intended to be run on samples of human (or mammalian) bodily fluids is not a satisfactorily informative test for identifying the presence of live *Legionella* bacteria--as application Serial No. 09/458,998 in fact teaches. In the application Serial No. 09/518,165 where the samples contemplated are in all instances human bodily fluids such as urine, blood, saliva, spinal fluid, and the like, extraction has not been found to be needed. See ¶10(c) above.

(j) The paragraph of the action bridging pages 9 and 10 of the action is not readily comprehensible to me. The specification of application 09/518,165 at p.2 under the heading "BACKGROUND OF THE INVENTION" states that

> "Gram-negative bacteria are known to have in common the possession of at least one lipo-polysaccharide or other lipo-carbohydrate antigen, while Gram-positive bacteria are known to possess the common characteristic of having at least one carbohydrate antigen that is a lipo-teichoic acid or a teichoic acid or a derivative of either."

This is a recitation of information that is well-known in the art of immunology and has been so known throughout my own career in the field.

The application says nothing about making derivatives from the crude target antigen, nor do I perceive any reason why it should do so. Applicants here are not in engaged in making derivatives of the target antigens or in substituting such derivatives for the carbohydrate antigens that are indigenous to a particular bacteria species or serogroup of a species. The invention contemplates *only* that an embodiment of the selected target carbohydrate antigen be rendered "essentially protein-free"--i.e. that its protein content be reduced to not more than 10% by weight and preferably less--and that that essentially protein-free antigen embodiment be applied to a chromatographic affinity column and used to purify antibodies which are then used to detect the crude embodiment of the same antigen in a sample of human bodily fluid. There is no occasion for "one of skill in the art...to perform experimentation to use derivatives of either lipoteichoic acid or teichoic acid to detect the crude antigen" as postulated at p.10 of the Office Action. *If* a target antigen of some bacteria species, or serogroup, should happen to be a *derivative* of either teichoic or lipoteichoic acid, there is no immunological reaction in which it could be used to detect itself. Furthermore, if a target carbohydrate antigen of some bacteria species, or serogroup of a species should be a derivative of teichoic acid or lipoteichoic acid, my experience and training in immunology tell me that the antibodies, whether raised against the bacteria or against an Ig G cut of the crude antigen, will be naturally adapted to be a binding partner of that derivative, so as to insure that the natural binding affinity between antigen and antibody will be preserved and that no one will need to "experiment" about how to use them to detect the crude antigen.

(k). The conclusion on page 12, "Accordingly, one of skill in the art would be required to perform undue experimentation to use esters of either lipoteichoic acid or teichoic acid to detect the crude antigen"

is a *non sequitur*. The application Ser. No. 09/518,165 does not disclose or contemplate that esters of lipoteichoic acid or teichoic acid could or would be utilized to "detect" *any* crude antigen. The mention of teichoic and lipoteichoic acids and their derivatives at pages 2 and 9 of this application clearly relate to all of them as *carbohydrate antigens*. Carbohydrate antigens are not usable, as anyone with minimal training in immunology knows, to "detect" other antigens.

Signed this 19 day of August 2003.

William J. Paliw